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Nanotechnologies for biomolecular detection and medical diagnostics

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Nanotechnology-based platforms for the high-throughput, multiplexed detection of proteins and nucleic acids in heretofore unattainable abundance ranges promise to bring substantial advances in molecular medicine. The emerging approaches reviewed in this article, with reference to their diagnostic potential, include nanotextured surfaces for proteomics, a two-particle sandwich assay for the biological amplification of low-concentration biomolecular signals, and silicon-based nanostructures for the transduction of molecular binding into electrical and mechanical signals, respectively.

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Introduction

As medicine steadily progresses toward diagnostics based on molecular markers, and highly specific therapies aimed at molecular targets, the necessity for high-throughput methods for the detection of biomolecules, and their abundance, concomitantly increases. Technology platforms that provide the reliable, rapid, quantitative, low-cost and multi-channel identification of biomarkers such as genes and proteins are *de facto* the rate-limiting steps for the clinical deployment of personalized medicine [1], in domains such as the early detection and the treatment of malignant disease. Early detection is particularly important in the case of cancer and other pathologies, because the early stages of disease are typically treated with the greatest probability of success.

The repeated screening of large populations for signs of precancerous developments, or the establishment of early malignant lesions is only conceivable in the context of the analysis of biological fluids such as blood, urine and sputum samples. To date, this has been impossible, largely because there are no contemporary approaches for the reliable, quantitative detection of multiple low-abundance protein markers, comprised within a formidable complexity of diverse biomolecular species in each bio-fluid specimen.

Nanotechnology offers promise, as a broad spectrum of highly innovative approaches emerges for the overcoming of this challenge [2,3•,4–11]. Four emerging approaches are reviewed below: nanostructured surfaces for the enhancement of proteomic analysis via mass spectrometry (MS) and reverse-phase protein microarrays; the bio-bar code method for the amplification of protein signatures via the use of two-particle, sandwich assay; nanowires as biologically gated transistors, transducing molecular binding events into real-time electrical signals; and silicon cantilevers for the mechanics-based recognition of biomolecular populations.

Nanostructured surfaces for proteomic analyses via MS and reverse-phase protein microarrays

MS is currently the gold standard for the protein expression profiling of biological fluids and tissues [12–14], with mounting evidence that matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS can be employed for the early detection of malignant disease. Current limitations of this approach include the complexity and reproducibility of the 2-D gel electrophoresis pre-fractionation steps required for its implementation on biological fluids. It is also recognized that the number of different biomolecular species in the plasma proteome probably exceeds 300 000, and could be as high as 10^6, with differences of as many as 12 orders of magnitude in

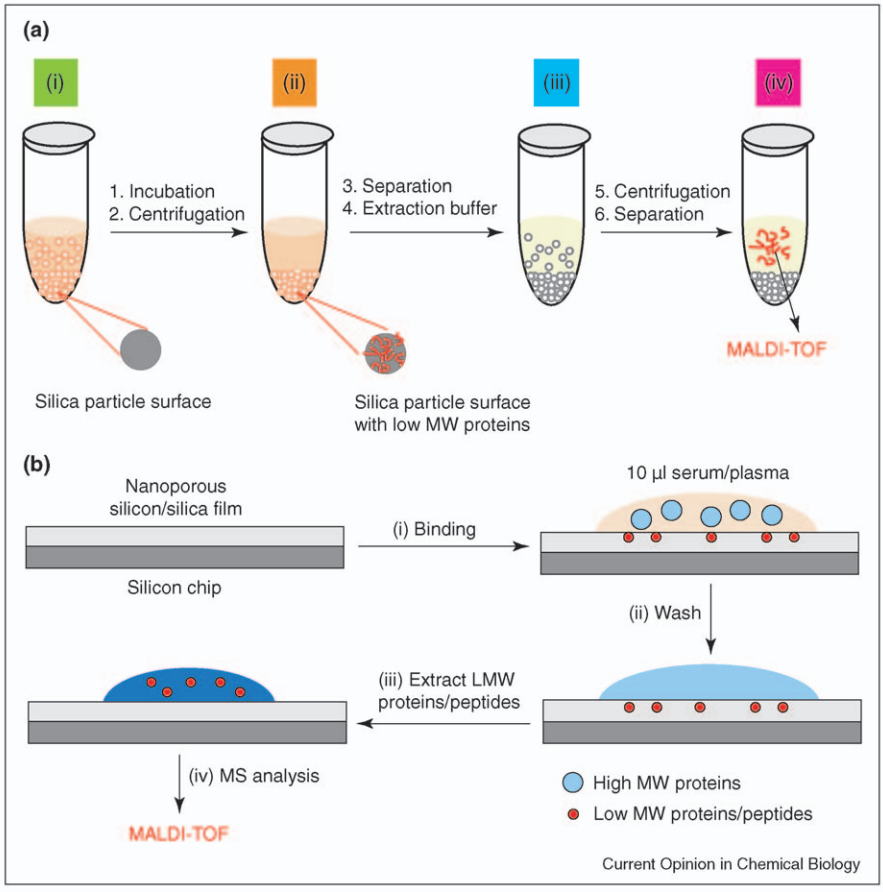
relative abundances. It is hypothesized that the low molecular weight proteome (LMWP), comprising proteolyic fragments at extremely low concentration, contains a wealth of information of diagnostic and prognostic utility [15]. Current MS methodologies do not enable the routine profiling of the LMWP.

The physico-chemical modification of nanoscale domains ('nanotexturing') on an MS planar or nanoparticle substrate has been proposed (Terracciano R *et al.* and Gaspari M *et al.*, unpublished data) with the objective of the size-exclusion-based, elective capture and enrichment of selected regions of the LMWP from body fluids. In proof-of-principle validation experiments, silicon oxide particles, obtained starting from silica gels, and silicon chips coated with a 500 nm-thick nanoporous film were challenged with a human plasma-diluted sample. MALDI-TOF analysis was then performed, as a means of detecting and assessing the extracted proteins (Figure 1).

Spiking experiments performed by adding peptide standards to human plasma at different concentrations showed that a MALDI-TOF signal can be detected from peptide amounts down to the ng/ml range (Figure 2). This demonstrates the effectiveness of silica platforms for increasing the sensitivity of MS analysis (roughly 400-fold). The selective enrichment achieved by the use of the different nanostructured surfaces depends on the pore size, the fabrication procedure and the experimental conditions, and represents a powerful tool to further improve the selectivity of peptide harvesting. Moreover, chemical and/or structural modifications of the surface of the silica substrate contribute to the 'tailoring' of the device for selective enrichment of specific protein/peptide classes.

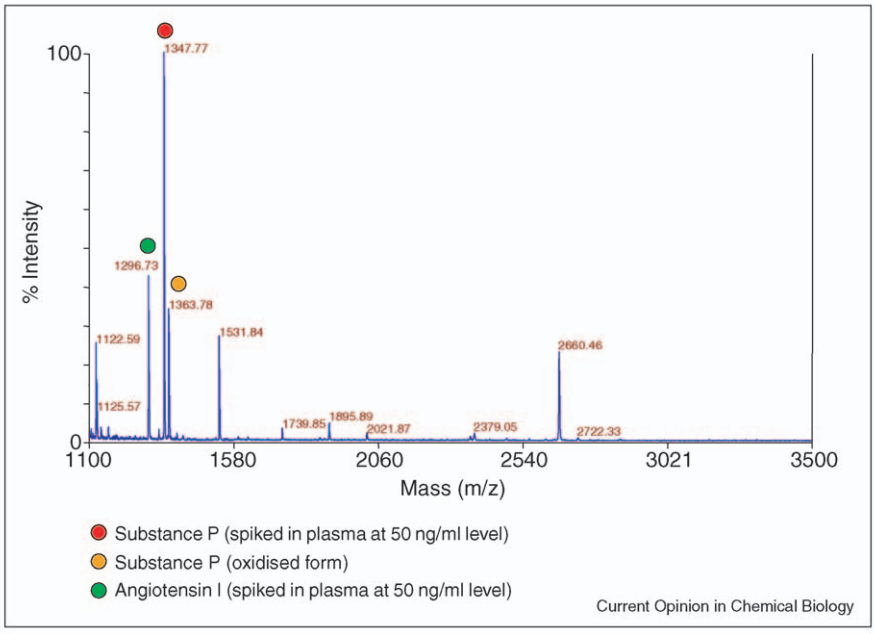
High-throughput MALDI-TOF MS analysis of LMW-enriched serum/plasma is an attractive strategy to rapidly and efficiently screen and profile a large number of samples, in the search for disease-related biomarkers.

Figure 1



Procedures for LMW plasma/serum protein harvesting. **(a)** Capturing strategy on silica nanoparticles. (i) Incubation of human plasma with silica nanoparticles and adsorption of LMW proteins; (ii) Centrifugation and separation of plasma from nanoparticles; (iii) Extraction of LMW proteins from nanoparticles; (iv) LMW proteins are recovered, followed by MALDI-TOF analysis. **(b)** Capturing strategy on nanoporous surfaces. (i) Incubation of serum/plasma with nanoporous silicon/silica film; (ii) Washing of unbound substances; (iii) Release of LMW proteins/peptides by appropriate extraction solutions; (iv) MS analysis.

Figure 2



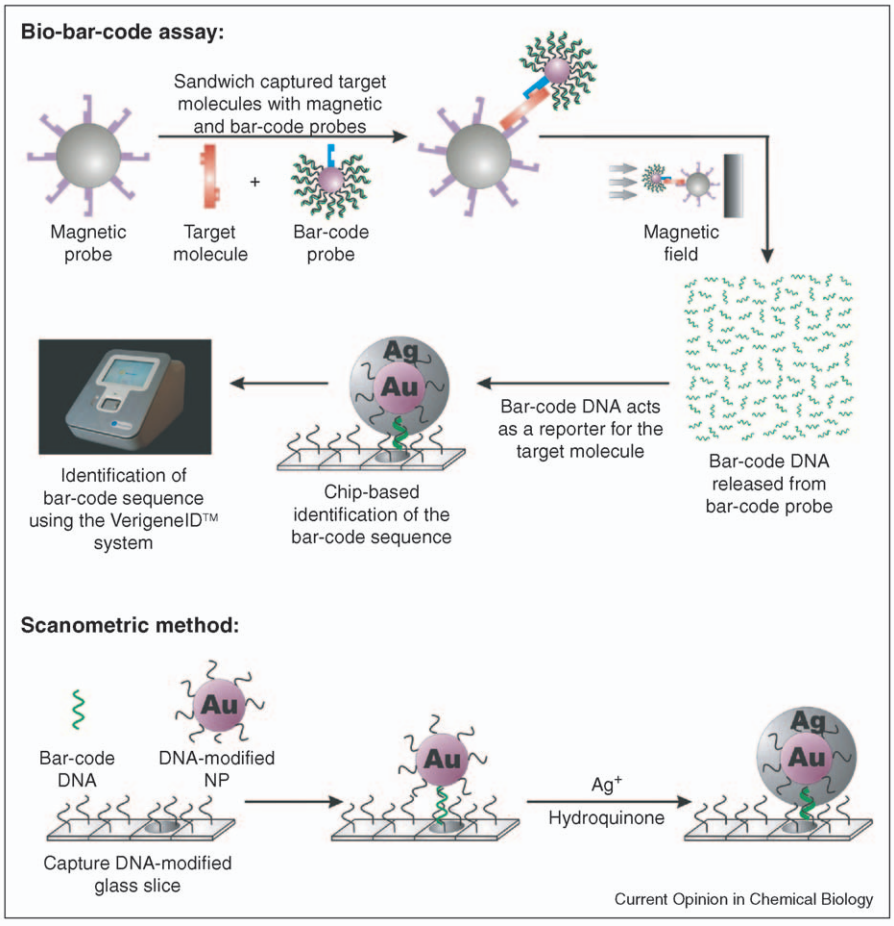
Mass spectrum (*m/z* range 1100–3500) of LMW peptides extracted by nanoporous harvesting of 10 μl of a human plasma sample. Standard peptides substance P and angiotensin I were spiked into plasma at 50 ng/ml level.

A second application of surface nanotechnology for biomolecular profiling pertains to reverse-phase protein microarrays (RPMA). These arrays enable the high-throughput screening of post-translational modifications of signaling proteins within diseased cells [16]. One limitation of protein-based molecular profiling is the lack of a PCR-like intrinsic amplification system for proteins. For this reason, the enhancement of microarray sensitivities is an important goal, especially because many molecular targets within patient tissues are of low abundance. Quantum dots are possible reporter agents because of their multiplexing potential and their potential for increased sensitivity [17]. The intrinsic fluorescent properties of nitrocellulose-coated glass slides limit the ability to image microarrays for extended periods of time where increases in net sensitivity can be attained. Silicon, with low intrinsic autofluorescence, has been explored as a potential microarray surface (Nijdam AJ *et al.*, unpublished data). Through semiconductor etching techniques, large surface areas can be created on silicon to enhance protein binding. Further, via chemical modification, reactive groups have been added to the surfaces. Using this combinatorial method of surface roughening and surface coating, the silicon surfaces were shown to transform native silicon into a protein-binding substrate comparable to nitrocellulose. The combination of equivalent protein-binding capabilities, with much lower autofluorescence indicates that nanotextured silicon may prove to be a superior alternative to nitrocellulose as an RPMA substrate.

The bio-bar-code assay

The bio-bar-code assay (Figure 3) is a powerful amplification and detection system for nucleic acids and proteins [3•,18–21]. It utilizes two types of particles to accomplish sample purification, detection and amplification. The first is a microparticle with a recognition agent. In the case of nucleic acids, the recognition agent is an oligonucleotide that is complementary to a statistically unique region of a target [19]. In the case of proteins, the recognition agent is a monoclonal antibody [3•]. The second particle is a nanoparticle with a recognition agent that can sandwich the target with the microparticle. The recognition agent can be either a polyclonal antibody in the case of a protein target or a complementary oligonucleotide in the case of a nucleic acid. In addition, the nanoparticle carries with it hundreds of oligonucleotides referred to as bar-codes. The bar-codes typically comprise 15–20-mer oligonucleotides, allowing the user to pair a unique bar-code with every conceivable recognition agent, since for a 20-mer, there are 4^{20} unique combinations. Once the two particles have sandwiched a target, a magnetic field can be used to separate the complexed target from the sample solution. Release of the barcodes in buffer is effected chemically (e.g. by dithiothreitol, DTT [20]) or by heating the solution [22–24], and the barcodes are then identified with a high sensitivity detection system. Thus far, scanometric [4] (Figure 3) and *in situ* fluorescence-based approaches have been used, but in principal any reasonable high sensitivity readout mechanism can be coupled to the system. The scanometric method has provided the

Figure 3

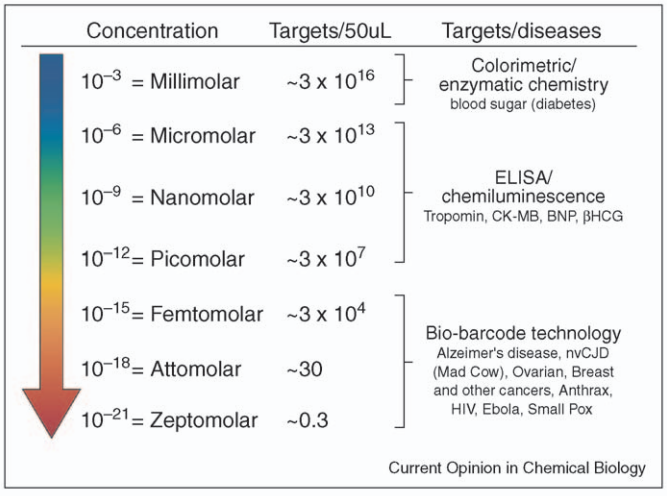


General bio-bar-code assay scheme. A magnetic probe captures a target using either monoclonal antibody or complementary oligonucleotide. Target-specific gold nanoparticles sandwich the target and account for target identification and amplification. The bar-code oligonucleotides are released, and detected using the scanometric method. The target can be DNA, RNA or Protein. The Verigene ID™ is a commercial instrument (Nanosphere Inc., Northbrook IL; http://www.nanosphere-inc.com).

lowest limit of detection to date for both nucleic acid (high zeptomolar, 10^{-21} M) [19] and protein targets (low attomolar, 10^{-18} M) [3•]. The speed and high sensitivity of the bio-bar-code assay derives from three unique features. First, it has very efficient, homogeneous target capture, as opposed to slow surface capture on a plate. Even with weak target-binding antibodies, the equilibrium can be pushed to complexation by increasing the concentration of the particle probes. Second, it has good amplification through a high ratio of barcode to target recognition element. Finally, it has high sensitivity bar-code sorting and sensing capabilities through the chip-based scanometric method.

The barcode detection system allows one to detect nucleic acids close to the sensitivity of PCR without the need for complicated enzymatic processes, and can be viewed as a potentially more efficient alternative to PCR that will soon be available for widespread use in research and clinical applications. Although alternatives to PCR are important, the barcode assay is likely to have its most significant scientific and clinical impact in protein marker-based diagnostics. It is up to 10^6 times more sensitive than ELISA-based technology, offering researchers and clinicians at least three major opportunities: (1) the ability to use new markers for diagnosing many types of diseases that could not be considered with conventional technology because of a lack of sensitivity; (2) the ability to look at known disease markers via less invasive means; and (3) the ability to use existing markers to evaluate disease recurrence. An example is the use of the barcode assay to identify amyloid-derived diffusible ligands (ADDL, a marker first linked to Alzheimer's disease through studies of the brain) in cerebral spinal fluid samples of subjects afflicted with the disease [21]. This was the first time the ADDLs were identified in fluids outside of the brain and, importantly, preliminary data showed a correlation between ADDL concentration

Figure 4



Biomolecule detection technology. The bio-barcode assay provides access to a target concentration range well below that of conventional ELISAs. This ultra-sensitivity provides the ability to utilize new markers for disease screening in biodiagnostics.

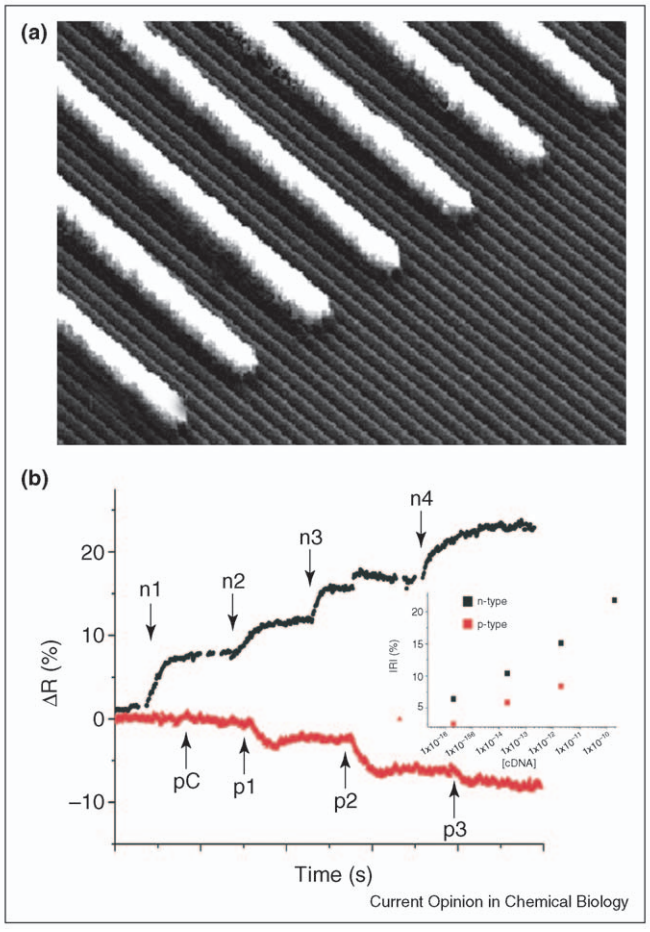
and progression of the disease. The barcode assay has also been used to develop high sensitivity serum-based prion detection systems for bovine spongiform encephalopathy ('mad cow disease'). Finally, it is being evaluated as a potential tool for studying recurrence in prostate cancer patients. The rationale for the application of the bar-code assays is that patients who have had their prostates surgically removed should have exceedingly low prostate specific antigen (PSA) levels (significantly below what can be detected with conventional diagnostic tools), and if the cancer is recurring, the barcode assay will be able to identify the rise in PSA well before conventional tests, thereby potentially increasing the breadth of successful treatment options. It is projected that the barcode assay and variations on it will redefine several aspects of modern biodiagnostics for many types of cancer, heart disease, HIV, and neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease (Figure 4).

Nanowires: label-free electronic sensors of genes and proteins

Nanowire sensors operate on the basis that the change in chemical potential accompanying a target/analyte binding event, such as DNA hybridization [8], can act as a field-effect gate upon the nanowire, thereby changing its conductance. This is similar, in principle, to how a field-effect transistor operates. The ideal nanowire sensor is a lightly doped, high-aspect ratio, single-crystal nanowire with a diameter between 10 nm and 20 nm. If it is much smaller, it will be too noisy a sensor, and if it is much larger, it is not as sensitive. Even exotic lithographic patterning methods, such as electron beam lithography, cannot produce such small width, high aspect ratio semiconductor structures [25]. Thus, materials methods have been employed to grow nanowires, which are then assembled, using various fluidics approaches, into individual devices. Most recently, a patterning method, called superlattice nanowire pattern transfer, or SNAP [26], has been demonstrated as capable of producing large arrays of silicon nanowires (Figure 5a) with excellent conductivity characteristics [27].

One compelling advantage of nanowire sensors is that the number and density of the sensor elements is limited only by the ability to electronically address individual nanowires. Very dense nanowire sensor circuits may be addressed. Thus, large-scale circuits can, in principle, be constructed within very small (microfluidics) environments, thereby enabling measurements of large numbers of different genes and proteins from very small tissue samples, or even single cells [1]. The potential for both biological research and clinical applications is large. However, encoding the individual nanowires with ssDNA molecules or protein capture agents represents a serious challenge. Electrochemical methods [28] have been applied to encoding the surfaces of electronically selected nanowires with proteins [9]. Such methods are, again, only limited by the ability to electrically address the nanowire sensors, and so can lead to very dense sensor libraries. For protein detection, biofouling will probably limit the ultimate library size that can be constructed. Also, for protein detection in typical environments such as serum or tissue culture media, either the ionic strength of the media must be greatly reduced, or small molecular protein capture agents must be utilized instead of antibodies. This is because Debye screening in 0.14 M electrolyte will effectively shield the nanosensor from

Figure 5



Silicon nanowire sensors for real time, electronically transduced, label-free biomolecular detection in electrolyte. **(a)** An electron micrograph of SNAP-fabricated silicon nanowire sensors. The nanowires in this image are 14 nm wide. For actual sensing measurements, the metal contacts (patterned by electron beam lithography) are coated with silicon nitride, varying numbers of nanowires (5–10) are contacted per sensing element, and the sensing circuit is entrained in a microfluidics environment. **(b)** Sensing results from n-type (top) and p-type Si nanowire sensors, demonstrating sensitivity in the attomolar (10^{-18} M) range in 1 × SSC buffer (0.15 M NaCl, 0.15 M sodium citrate, pH 7.0). The *y*-axis (time) is 0–600 s for n-type sensors and 0–1400 s for p-type sensors. The nanowire sensing elements were coated with ssDNA, and complementary (or non-complementary) ssDNA oligonucleotides, in 0.15 M electrolyte, were flowed over the nanosensors using microfluidics. The various points indicated are: n1 = 220 attoM cDNA; n2 = 22 femtoM cDNA; n3 = 2.2 pM cDNA; n4 = 220 pM cDNA; pC = 22 nanoM non-complementary ssDNA; p1 = 220 attoM cDNA; p2 = 22 femtoM cDNA; p3 = 2.2 pM cDNA. Inset: Both p- and n-type nanosensors exhibit sensitivity over a broad dynamic range, although their response scales logarithmically with concentration.

detecting the protein/antibody binding event for typical (large molecular weight) antibodies.

Images of, and data from a silicon nanowire sensor library, prepared using the SNAP process, are reported in Figure 5. The data demonstrate the broad dynamic range (10^6) of sensing the nanowires can attain. They also indicate the selectivity of these sensors and the possibility of using p- and n-type devices as a sensing pair for increased signal to noise. Libraries containing up to 24 individual nanowire sensors have been constructed, and it should be possible to extend these libraries to 10^3–10^5 elements [29], for the purpose of rapid, high-throughput, highly multiplexed biomolecular detection.

Cantilevers: nanomechanical detection of biological molecules

Detection of extremely small forces using micro- and nanoelectromechanical systems (MEMS and NEMS) is well established. Recently, it has been demonstrated that molecular adsorption also results in measurable mechanical forces. Detecting biomolecular interactions by measuring nanomechanical forces offers an exciting opportunity for the development of highly sensitive, miniature and label-free biological sensors [11,30]. For example, micron-sized silicon cantilever beams undergo bending due to surface stresses created by molecular adsorption, when adsorption is confined to a single side of the cantilever. The cantilever beam amplifies the

forces involved in the adsorption process into nanometer displacement. A cantilever's resonance frequency also varies as a function of molecular adsorption due to mass loading [31]. The adsorption-induced bending and frequency variation can be measured simultaneously by using several techniques, such as variations in optical beam deflection, piezoresistivity, piezoelectricity and capacitance. Although these motion detection methods are extensively used with cantilevers, micron and sub micron cantilevers will require techniques more suitable for nanoscale measurements such as electron tunneling. Because of recent advances in lithographic technologies and micro- and nano-fabrication techniques, these sensors can be mass-manufactured on silicon wafers and other materials in a cost-effective and modular fashion.

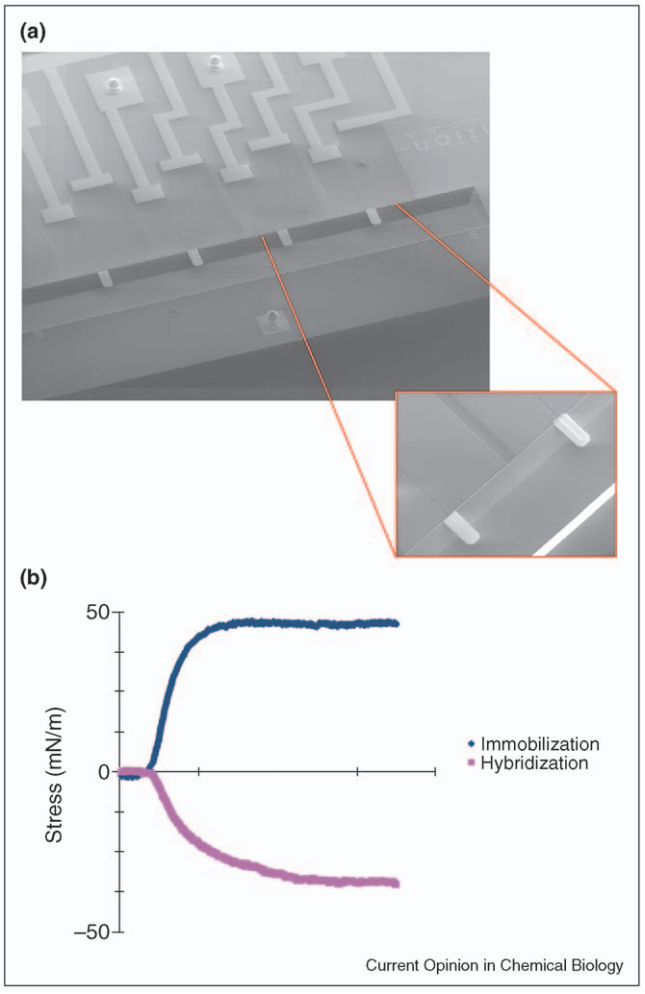
Although cantilever sensors are extremely sensitive, they offer no intrinsic chemical selectivity. Selective chemical recognition is achieved by affinity binding reactions, where the cantilever is coated with self-assembled monolayers, DNA probes, antibodies or peptides. The cantilever-bending mechanical stresses originate from the free-energy changes induced by specific biomolecular binding. Non-specific interactions of biomolecules do not cause cantilever bending (Figure 6).

DNA hybridization on the cantilevers has been extensively investigated by several groups [32,33]. Thiol-modified ssDNA probes (20-mers) were immobilized on one side of a cantilever with a vacuum-deposited gold layer of 40 nm thickness. When complementary ssDNA targets were injected into the liquid cell holding the cantilever array, the cantilevers with DNA probes underwent bending caused by hybridization. The extent of cantilever bending varied as a function of the length of the complementary ssDNA. The cantilever deflection was attributed to reduction in surface stress due to conformational changes caused by double-stranded DNA formation (Figure 6).

The detection of proteins by cantilever nanomechanics appears to be more challenging, mainly because of the lack of reproducible and robust immobilization techniques for antibodies. Antigens that have been successfully detected on cantilevers using immobilized antibodies include PSA, and the biowarfare agents ricin and tularaemia [34,35]. Upon exposure to antigens, the antibody-immobilized cantilevers undergo bending, with bending amplitude proportional to concentration and time of exposure. The biomolecular interaction-induced cantilever bending is irreversible at room temperature.

Mechanical label-free detection, although still in its early days, has the potential as a platform for sensitive, multiplexed sensors for biomolecules. Currently available micromachining technologies could be used to make multi-target sensor arrays involving tens of cantilevers

Figure 6



Nanocantilevers for ssDNA detection. **(a)** An array of piezoresistive cantilevers in a fluidic well (Cantion Inc.). The cantilevers are 120 microns long and are separated by 470 microns. **(b)** Cantilever surface stress variation as a function of ssDNA probe (20-mers) immobilization and hybridization of fully complementary 20-mers using a piezoresistive cantilever array (taken with a reference cantilever).

and analog processing on a single chip. Increasing the number of sensing elements in an array can lower noise, increase selectivity, and enhance robustness. Simplicity, low power consumption, potential low cost, inherent compatibility with array designs, and label-free detection make cantilever sensors very attractive for a variety of applications.

Several challenges must be overcome before cantilever array sensors can come into widespread use. More advances are needed in developing efficient immobilization techniques that can transduce the stress involved in biochemical interaction to the cantilever substrate. Advances in nanofabrication and nanoscale motion detection are essential for the viability of nanomechanical

detection platforms. Technology for designing electronic chips is well advanced, but integration of electronic, mechanical and fluidic designs is still in its infancy, and efforts are underway to accelerate the design of fully integrated devices.

Conclusion

Nanotechnology-based platforms offer promise for the attainment of multiple elusive goals in biomolecular analysis. Primary examples of emerging approaches include surface nanotexturing for MS and RPMAs, the bio-bar code assay, biologically gated nanowire sensors, and nanomechanical devices such as bio-derivatized cantilever arrays. Through their individual or combined uses it is envisioned that progress will be accomplished in the high-throughput multiplexing of analyses of nucleic acids and proteins, resulting in commensurate advances in medical diagnostics.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hood L, Heath JR, Phelps ME, Lin B: **Systems biology and new technologies enable predictive and preventative medicine**. *Science* 2004, **306**:640-643.

2. Ferrari M: **Cancer nanotechnology: opportunities and challenges**. *Nature Rev Cancer* 2005, **5**:161-171.

3. • Nam J-M, Thaxton CS, Mirkin CA: **Nanoparticle-based bio-bar codes for the ultrasensitive detection of proteins**. *Science* 2003, **301**:1884-1886.
Original paper introducing the Bio-Bar-Code assay, which demonstrates its capability to detect low-attomolar protein concentrations in solution using DNA-functionalized gold nanoparticles.

4. Taton TA, Mirkin CA, Letsinger RL: **Scanometric DNA array detection with nanoparticle probes**. *Science* 2000, **289**:1757-1760.

5. Lasseter TL, Cai W, Hamers RJ: **Frequency-dependent electrical detection of protein binding events**. *Analyst* 2004, **129**:3-8.

6. Fritz J, Baller MK, Lang HP, Rothuizen H, Vettiger P, Meyer E, Guntherodt HJ, Gerber C, Gimzewski JK: **Translating biomolecular recognition into nanomechanics**. *Science* 2000, **288**:316-318.

7. Cui Y, Wei Q, Park H, Lieber CM: **Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species**. *Science* 2001, **293**:1289-1292.

8. Hahm J.-i., Lieber CM: **Direct ultrasensitive electrical detection of DNA and DNA sequence variations using nanowire nanosensors**. *Nano Lett* 2004, **4**:51-54.

9. Bunimovich YL, Ge G, Beverly KC, Ries RS, Hood L, Heath JR: **Electrochemically programmed, spatially selective biofunctionalization of silicon wires**. *Langmuir* 2004, **20**:10630-10638.

10. Beckman R, Johnston-Halperin E, Luo Y, Green JE, Heath JR: **Bridging dimensions: demultiplexing ultrahigh-density nanowire circuits**. *Science* 2005, **310**:465-468.

11. Thundat T, Majumdar A: **Microcantilevers for physical, chemical, and biological sensing**. *Sensors Sensing Biol Eng* 2003:338-355.

12. Wright ME, Han DK, Aebersold R: **Mass spectrometry-based expression profiling of clinical prostate cancer**. *Mol Cell Proteomics* 2005, **4**:545-554.

13. Pan S, Zhang H, Rush J, Eng J, Zhang N, Patterson D, Comb MJ, Aebersold R: **High throughput proteome screening for biomarker detection**. *Mol Cell Proteomics* 2005, **4**:182-190.

14. Stoeckli M, Chaurand P, Hallahan DE, Caprioli RM: **Imaging mass spectrometry: a new technology for the analysis of protein expression in mammalian tissues**. *Nat Med* 2001, **7**:493-496.

15. Liotta LA, Ferrari M, Petricoin E: **Clinical proteomics: written in blood**. *Nature* 2003, **425**:905.

16. Liotta LA, Espina V, Mehta AI, Calvert V, Rosenblatt K, Geho D, Munson PJ, Young L, Wulfkuhle J, Petricoin EF III: **Protein microarrays: meeting analytical challenges for clinical applications**. *Cancer Cell* 2003, **3**:317-325.

17. Geho D, Lahar N, Gurnani P, Huebschman M, Herrmann P, Espina V, Shi A, Wulfkuhle J, Garner H, Petricoin E III *et al.*: **Pegylated, streptavidin-conjugated quantum dots are effective detection elements for reverse-phase protein microarrays**. *Bioconjugate Chemistry* 2005, **16**:559-566.

18. Nam J-M, Park S-J, Mirkin CA: **Bio-barcodes based on oligonucleotide-modified nanoparticles**. *J Am Chem Soc* 2002, **124**:3820-3821.

19. Nam J-M, Stoeva SI, Mirkin CA: **Bio-bar-code-based DNA detection with PCR-like sensitivity**. *J Am Chem Soc* 2004, **126**:5932-5933.

20. Thaxton CS, Hill HD, Georganopoulou DG, Stoeva SI, Mirkin CA: **A biol-bar-code assay based upon dithiothreitol-induced oligonucleotide release**. *Anal Chem* 2005, **77**:8174-8178.

21. Georganopoulou DG, Chang L, Nam J-M, Thaxton CS, Mufson EJ, Klein WL, Mirkin CA: **Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease**. *Proc Nat Acad Sci USA* 2005, **102**:2273-2276.

22. Mirkin CA, Letsinger RL, Mucic RC, Storhoff JJ: **A DNA-based method for rationally assembling nanoparticles into macroscopic materials**. *Nature* 1996, **382**:607-609.

23. Elghanian R, Storhoff JJ, Mucic RC, Letsinger RL, Mirkin CA: **Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles**. *Science* 1997, **277**:1078-1080.

24. Park SJ, Taton TA, Mirkin CA: **Array-based electrical detection of DNA with nanoparticle probes**. *Science* 2002, **295**:1503-1506.

25. Vieu C, Carcenac F, Pepin A, Chen Y, Mejias M, Lebib A, Manin-Ferlazzo L, Couraud L, Launois H: **Electron beam lithography: resolution limits and applications**. *Appl Surface Sci* 2000, **164**:111-117.

26. Melosh NA, Boukai A, Diana F, Gerardot B, Badolato A, Petroff PM, Heath JR: **Ultrahigh-density nanowire lattices and circuits**. *Science* 2003, **300**:112-115.

27. Beckman RA, Johnston-Halperin E, Luo Y, Melosh N, Green J, Heath JR: **Fabrication of Conducting Silicon Nanowire Arrays**. *J Appl Phys (communication)* 2004, **96**:5921-5923.

28. Yousaf MN, Mrksich M: **Diels-Alder reaction for the selective immobilization of protein to electroactive self-assembled monolayers**. *J Am Chem Soc* 1999, **121**:4286-4287.

29. Beckman R, Johnston-Halperin E, Luo Y, Green JE, Heath JR: **Bridging dimensions: demultiplexing ultrahigh-density nanowire circuits**. *Science* 2005, **310**:465-468.

30. Ziegler C: **Cantilever-based biosensors**. *Anal Bioanal Chem* 2004, **379**:946-959.

31. Lee JH, Hwang KS, Park J, Yoon KH, Yoon DS, Kim TS: **Immunoassay of prostate-specific antigen (PSA) using**

resonant frequency shift of piezoelectric nanomechanical microcantilever. *Biosensors Bioelectronics* 2005, **20**:2157-2162.

32. Mukhopadhyay R, Lorentzen M, Kjems J, Besenbacher F: **Nanomechanical sensing of DNA sequences using piezoresistive cantilevers**. *Langmuir* 2005, **21**:8400-8408.

33. Alvarez M, Carrascosa LG, Moreno M, Calle A, Zaballos A, Lechuga LM, Martinez AC, Tamayo J: **Nanomechanics of the formation of DNA self-assembled monolayers and hybridization on microcantilevers**. *Langmuir* 2004, **20**:9663-9668.

34. Weeks BL, Camarero J, Noy A, Miller AE, Stanker L, De Yoreo JJ: **A microcantilever-based pathogen detector**. *Scanning* 2003, **25**:297-299.

35. Ji HF, Yang X, Zhang J, Thundat T: **Molecular recognition of biowarfare agents using micromechanical sensors**. *Expert Rev Mol Diagnostics* 2004, **47**:859-866.